

## Supporting Information

### Appendix S1

#### *Experimental Procedures*

##### *Mouse experiments*

The mtDNA mutator mice were derived from the Larsson laboratory (Trifunovic *et al.* 2004), backcrossed in Stockholm onto the C57Bl/6 background for 10 generations and maintained as heterozygous crosses with wild-type females from another colony, in order to avoid any impact of maternally transmitted mtDNA mutations. The mice used in this study homozygously and ubiquitously expressed the knocked-in mutated version of mitochondrial DNA polymerase  $\gamma$  containing a single point mutation (D257A) in the proof-reading 3'-5' exonuclease domain. Control mice were littermates homozygous for the wild type allele. Mice were maintained at 21 °C with free access to water and standard lab chow, and powdered food was added for all mice at age 25 weeks or older. When assessed with MitoB young mutator mice were 6 – 19 weeks of age and mature mutator mice were 35 – 42 weeks of age. The age-matched control mice were used at 6-20 weeks of age for young, 34-45 weeks for mature and 111 weeks for old. Data from both sexes are pooled for each group; sexes were not assessed separately due to insufficient numbers for comparison.

We measured mitochondrial hydrogen peroxide *in vivo* using the MitoB mass spectrometric probe as described previously (Cocheme *et al.* 2011; Cocheme *et al.* 2012; Chouchani *et al.* 2013). Briefly, 75 nmol MitoB ( $\sim 3 \mu\text{mol kg}^{-1}$  for 25 – 30 g mice) in 50  $\mu\text{l}$  saline was administered by tail vein injection to the mouse by restraining the mouse. Injections took place between 10 am and 12 noon and the mice were returned to their cages and returned to normal levels of activity for 6 h. At the end of this time the mice were killed by cervical dislocation and tissues (heart, liver, kidney and skeletal muscle (gastrocnemius)) were harvested and flash frozen on liquid nitrogen. To do this the tissue was dissected and within 1 sec was flash frozen between two aluminum blocks held at -190 °C with liquid N<sub>2</sub>. This thin layer of tissue was then kept immersed in liquid nitrogen in a small mortar until all tissues were dissected and similarly flash frozen. Then all tissues were wrapped in pre-labelled aluminum foil and placed in liquid nitrogen. Tissues were then stored at -80°C and transported to Cambridge, UK on dry ice for analysis. Blood was taken from the hearts of mice just immediately after euthanasia, proteinase inhibitors were added and serum was frozen and stored at -80°C. For the analysis of cytokines, the mice were injected intraperitoneally with 20  $\mu\text{g}$  of LPS (*Escherichia coli* 055:B5 from Sigma L6529-1MG) in 100  $\mu\text{l}$  sterile PBS and compared with mice that were injected with saline alone.  $187 \pm 35$  minutes (mean  $\pm$  sd,  $n = 25$ ) after injection the mice were euthanized and plasma samples isolated and analysed for cytokine content. All experiments were approved by the animal ethics committee of the North Stockholm region (N31/12).

#### *Analysis of tissue levels of MitoB and MitoP*

MitoB and MitoP were extracted from tissues and assessed by LC-MS/MS as previously described (Cocheme *et al.* 2011; Cocheme *et al.* 2012; Chouchani *et al.* 2013). Briefly, ~50 mg tissue were homogenised, spiked with deuterated internal standards, and MitoB and MitoP were extracted using acetonitrile/formic acid. MitoP and MitoB were detected using an I-class Acquity LC attached to a Xevo TQ-S triple quad mass spectrometer (Waters), analysed using MassLynx software, and the amounts of MitoP and MitoB in each sample was determined relative to a standard curve. The MitoP/MitoB ratios for each sample were then calculated. One of the 8 mature control mice showed anomalously high levels of MitoB in the liver, kidney and heart (3.5, 1.8 and 2.6-fold greater than the means of the other 7 mice in this cohort, with the increases over the means 5.9x, 3.4x and 6.9x of the standard deviation of the rest of the cohort). The MitoB injection for this mouse was therefore judged to be unreliable and data from this mouse were excluded from the analysis.

#### *Analysis of citrate synthase*

Tissues, that had been stored frozen until required, were homogenised in 50 mM Tris-HCl, 2 mM sodium citrate, 0.6 mM MnCl<sub>2</sub>, pH 7 and stored frozen until required. Citrate synthase was assessed as described (Robinson Jr. *et al.* 1987). Protein concentration was determined by the BCA assay.

#### *Analysis of serum cytokines*

Plasma cytokine levels were measured by the Core Biochemical Analysis Laboratory at Addenbrooke's Hospital, Cambridge, using a multiplexed electrochemical luminescence immunoassay on an automated biochemical analyser (MesoScale Discovery Sector 6000 analyser, Gaithersburg, MD, USA). Samples were analysed using a 7-plex proinflammatory cytokine high sensitivity kit (product code K15012C-2, MesoScale Discovery) which evaluated interleukin IL-1 $\beta$ , IL-6, IL-10, IL-12, tumour necrosis factor alpha (TNF- $\alpha$ ), interferon gamma (IFN $\gamma$ ) and chemokine KC/GRO. All reagents and calibration standards were supplied by the manufacturers. A stock solution of recombinant cytokines was used to produce a standard curve against which sample concentrations were determined.

## Supplementary References

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Supplementary Figure 1

